

Supplementary Information

Accessing Biominerals from By-Products Wasted by the Seafood Processing Industry

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1. Equations to determine crystallinity

Equation S1. Crystallinity =
$$\frac{I_{300} - V_{112/300}}{I_{300}}$$

- I_{300} : Intensity of (3 0 0) diffraction peak of hydroxyapatite (HAP)
- $V_{112/300}$: Reflection of the hollow between (1 1 2) and (3 0 0) diffraction peaks of HAP
- Taken and modified from (1) M. Z. U. A. Mamun, M. S. Hossain, S. P. Moulick, M. Begum, R. A. Sathee, M. S. Hossen, F. Jahan, M. M. Rashid, F. Islam, R. H. Bhuiyan and M. S. Alam, *Heliyon*, 2023, **9**, e18012., (2) H. B. Modolon, J. Inocente, A. M. Bernardin, O. R. Klegues Montedo and S. Arcaro, *Ceram. Int.*, 2021, **47**, 27685–27693., (3) E. S. Akpan, M. Dauda, L. S. Kuburi, D. O. Obada and D. Dodoo-Arhin, *Res. Phys.*, 2020, **17**, 103051.

Equation S2. Crystallinity =
$$\frac{C}{A + C}$$

- C : Sum of peaks area
- A : Area between peaks and background
- Taken from F. Carella, M. Seck, L. D. Esposti, H. Diadiou, A. Maienza, S. Baronti, P. Vignaroli, F. P. Vaccari, M. Iafisco and A. Adamiano, *J. Environ. Chem. Eng.*, 2021, **9**, 104815.

Equation S3. Crystallinity =
$$\frac{AC}{AT}$$

- AC : Area of crystalline peaks
- AT : Total area of amorphous and crystalline peaks
- Taken from P. Injorhor, T. Trongsatitkul, J. Wittayakun, C. Ruksakulpiwat and Y. Ruksakulpiwat, *Polymers*, 2022, **14**, 4158.

2. Experimental details for Figure 6

Procedure for hydroxyapatite nanoparticle preparation:

Briefly, salmon frames were manually cleaned of excess meat, blended for 1 min, boiled for 1 h in tap water, and enzymatically treated with 15 $\mu\text{L g}^{-1}$ Neutrase and 7.5 $\mu\text{L g}^{-1}$ Lipozyme CALB L for 6 h in water at 40 °C. The bones were allowed to dry in air overnight before being pulverized in a ball mill for 1 h with cooling breaks after every 20 min to prevent overheating.

Samples subjected to ultrasound were treated by dispersing 10 mg bone powder in 10 mL of 10% propanoic acid. The solution was sonicated for 15 min with a Misonix S-4000 sonicator employed with a circulating cooling bath set to 3 °C to prevent temperature fluctuations. The amplitude of the ultrasonic vibration was set to 50%. This process was repeated under identical conditions for several samples to ensure reproducibility. Next, the mixture was centrifuged for 15 min at 6000 rpm. The supernatant was decanted and retained for TEM analyses while the residual pellet was discarded.

Transmission electron microscope:

Negatively stained samples were analysed using a transmission electron microscope (TEM, HITACHI H-7500, Japan) in bottom-mounted contrast mode. TEM grids (copper 200 mesh, 12–25 nm carbon supported, Ted Pella Inc.) were freshly glow-discharged using an EMS GloQube-D, dual chamber glow discharge system (Electron Microscopy Sciences, PA) in negative mode with a plasma current of 25 mA for 45 s. These grids were floated on 10 μL sample aliquots on Parafilm for 2 min. The excess droplets were subsequently wicked away from the edge of the grid with filter paper strips (Whatman™ 541). The grid was then rinsed with droplets of double distilled water. Immediately after rinsing with water, the grid was exposed to 10 μL of Van Gieson's staining solution for 60 s and the stain was carefully removed using a fresh piece of filter paper. Finally, the grid was dried under ambient conditions for 2 h and used for TEM analysis.